CLAIM + DETAILED DESCRIPTION

[Claim(s)]
[Claim 1]
The following (a) or protein of (b);
(a) Protein which becomes SEQ ID NO 2 from an amino acid sequence of a description;
(b) An immunosuppressive receptor which amino acid becomes from deletion and an amino acid sequence replaced or added in an amino acid sequence of SEQ ID NO 2.
[Claim 2]
DNA given in either of the following (a) - (c);
(a) DNA which becomes SEQ ID NO 1 from a base sequence of a description;
(b) DNA which hybridizes on DNA of SEQ ID NO 1, and stringent conditions, and encodes an immunosuppressive receptor;
(c) DNA which encodes protein of a description in Claim 1
[Claim 3]
A recombination vector containing the DNA according to claim 2.
[Claim 4]
A transformed cell transformed by the recombination vector according to claim 3.
[Claim 5]
Antisense nucleic acid which controls a manifestation of the protein according to claim 1.
[Claim 6]
The antisense nucleic acid according to claim 5 whose base sequence is all or arrangement which carries out the complementation in part of nucleic acid of the DNA according to claim 2.
[Claim 7]
An antibody to protein according to claim 1 or its partial peptide.
[Claim 8]
A search method of a substance in which activity accommodation of this protein is shown contacting a transformed cell which has revealed the protein according to claim 1 or this protein, and an examined substance.
[Claim 9]
A search method of a substance in which an expression control operation of the DNA according to claim 2 is shown contacting a recombination vector, or a transformed cell according to claim 4 according to claim 3 and an examined substance.
[Claim 10]

A mcd055 gene-recombination nonhuman animal.

[Detailed Description of the Invention]
[0001]
[Field of the Invention]
DNA in which this invention encodes a new immunosuppressive receptor and its partial peptide, and this protein, and its fragment, It is related with the antibody and gene recombination nonhuman animal which have reactivity in the search method of the substance in which the activity accommodation of a transformed cell and this protein transformed by the recombination vector containing this DNA and this recombination vector is shown, this protein, or its partial peptide.
[0002]
[Description of the Prior Art]
By communicating with other cells, the cell is controlling the function and multiplication of a cell, and specialization. The cell adhesion molecule of the immunocompetent cell which participates in all immunoreactions in the living body deeply also in a cell adhesion molecule (cellular adhesion molecule) is an important thing which adjusts the immune function itself.
[0003]
The immunoglobulin domain is found out by very much protein by the immune system and the nervous system.
It is found out also in much other protein which participates in a cell-cell recognition mechanism.
These molecules are named generically and it is called immunoglobulin superfamily.
Although many cell adhesion molecules with immunoglobulin domain structure are known, IL-1 receptor, gp130, a PDGF receptor, etc. are mentioned, for example.
[0004]
ITIM (Immunoreceptor Tyrosine based Inhibition Motif) is a motif accepted in common with an immunosuppressive receptor, . [the phosphatase or SH2 domain which will be called SHP1 (SH2-containing protein tyrosine phosphatase-1) and SHP2 if the tylosin residue contained in this motif is phosphorylated] It is known that lipid phosphatase SHIP (SH2-containing inositol polyphosphate 5-phosphatase) which it has will join together. This phosphatase carries out dephosphorization of tyrosine kinases, such as syk, or a lipid like IP_4 or PIP_3. As a result, it is thought by controlling the Ca ion outflow from Ca store, and the Ca ion inflow from the outside of a cell that a negative signal is caused.
[0005]
Although CTLA-4, FcgammaRIIB, Killer cell inhibitory receptor (KIR), CD22, etc. are known, [the immunosuppressive receptor belonging to immunoglobulin superfamily] For the elucidation of a complicated immunoreaction in the living body and inflammatory reaction, isolation identification of a new immunosuppressive receptor which participates in these is desired.
[0006]
[Problem to be solved by the invention]
This invention provides prevention and treatment of immunopathy with useful physic and

procedure by identifying a new immunosuppressive receptor and its gene.
[0007]
[Means for solving problem]
This invention relates to the new immunosuppressive receptor (MCD055) by which a code is carried out to a new gene (referred to as mcd055), and the gene concerned. It is related with the antibody and gene recombination nonhuman animal which have reactivity in the search method of the activity pacemaker of the transformed cell transformed by the recombination vector containing this DNA and this recombination vector, and this protein, this protein, or its partial peptide.
[0008]
(Nucleic acid)
This invention provides gene mcd055 which encodes MCD055 (it states in detail later). Gene mcd055 is DNA which encodes the immunosuppressive receptor which specifically consists of an amino acid sequence shown in SEQ ID NO 2. Although cDNA shown in SEQ ID NO 1 or SEQ ID NO 3 as an example is mentioned, it is not limited to these, and the genomic DNA in which this cDNA besides cDNA shown in SEQ ID NO 1 or SEQ ID NO 3 originates is also contained. Although isolation identification of this gene can be carried out from the Homo sapiens spleen, it may be DNA prepared by the chemical synthesis procedures using the genetic engineering procedures, such as general hybridization based on the arrangement indicated by this Description, such as cloning and a phospho aminodite method. Although chemical synthesis DNA besides cDNA and genomic DNA etc. is contained as the form, there is no restriction in particular. Even if DNA of this invention is a single strand, it may combine with DNA and RNA which have complementary arrangement, and it may form a double chain and 3 heavy chain in it. The sign of the DNA concerned may be carried out by enzyme and radioisotopes, such as horseradish peroxidase (HRP), the fluorescent substance, the chemiluminescence substance, etc.
[0009]
Since the arrangement of RNA drawn from this, the arrangement of complementary DNA and RNA, etc. will be uniquely determined if the base sequence of mcd055 is provided, [this invention] It should be understood as DNA of RNA corresponding to DNA of this invention, or this invention, and the thing which also provides DNA and RNA which have complementary arrangement. In this Description, "DNA" is synonymous with a "polynucleotide."
[0010]
DNA which becomes SEQ ID NO 1 from the base sequence of a description, and DNA hybridized on stringent conditions are also included in DNA of this invention.
[0011]
If the protein by which hybridizes on this and stringent conditions to DNA which becomes SEQ ID NO 1 from the base sequence of a description, and a code is carried out to this DNA is an immunosuppressive receptor, the variation of a base sequence is permitted. For example, existence of two or more codon which encodes the same amino acid residue what is called by codon degeneracy, With variation, deletion, a juncture, etc. of the DNA fragment by the random variation by various artificial processings, for example, site-specific variation introduction, and variation agent processing, and restriction enzyme cutting, If it is DNA which these DNA variant hybridizes under DNA

of a description, and stringent conditions to SEQ ID NO 1, and encodes an immunosuppressive receptor even if DNA arrangement changes partially, It is not concerned with the difference with the DNA arrangement shown in SEQ ID NO 1, but is a thing of this invention within the limits.

[0012]

If the grade of the above-mentioned DNA variation has not less than 98% of identity still more preferably not less than 95% preferably not less than 90% with the DNA arrangement of a description in SEQ ID NO 1, it will be in a tolerance limit. BLAST (J. Mol.Evol. and Vol.36;290-300 (1993), J.Mol.Biol., and Vol.215;403-10 (1990)) can be used for judgment of the identity of DNA arrangement. Under a condition stringent as a grade to hybridize, When [for example,] the label of the probe is carried out by DIG DNA Labeling kit (Roche Diagnostics Cat No.1175033), For example, 37 ** 32 ** is made to hybridize in 42 ** DIG Easy Hyb solution (Roche Diagnostics Cat No.1603558) more preferably, For example, the conditions which wash 50 ** of membrane preferably in 65 ** 0.5xSSC solution (SDS is included 0.1% (w/v)) (0.15 M NaCl) [1xSSC] What is necessary is just a grade hybridized to nucleic acid given in SEQ ID NO 1 by the Southern hybridization of being 0.015M sodium citrate.

[0013]

It is thought that DNA which becomes SEQ ID NO 1 from a base sequence of a description, or its partial fragment is useful as a specific probe of a disease with which protein of this inventions, such as an autoimmune disease, immune disorder, an allergic disease, an inflammatory disease, and a tumor, involves.

[0014]

DNA of this invention can be used in order to produce MCD055 in large quantities. The sign of this DNA is carried out with enzyme etc., and it can be used in order to inspect a manifestation situation of protein of this invention in an organization. [namely by using this DNA as a probe and checking the amount of mRNA manifestations for the amount of manifestations of protein of this invention in a cell as an index] A cell suitable for manufacture of protein of this invention and its culture condition can be determined, and also it is possible to diagnose a disease to which protein of this invention relates especially an autoimmune disease, immune disorder, an allergic disease, an inflammatory disease, a tumor, etc.

[0015]

The PCR-RFLP (Restrictionfragment length polymorphism) method which uses a part of DNA of this invention as a primer, PCR-SSCP (Single strand conformation polymorphism) -- abnormalities or polymorphism on a nucleic acid sequence can be inspected and diagnosed by procedures, such as law and seek ENSHINGU.

[0016]

DNA of this invention can be introduced into a cell in the living body, and it can be used also for the gene therapy for preventing or treating the onset of an autoimmune disease, immune disorder, an allergic disease, an inflammatory disease, a tumor, etc.

[0017]

DNA of this invention is substantially useful to the search of a compound which controls specifically preparation of a transformed cell, the production procedure of recombination protein MCD055 using this transformed cell further, or the manifestation of MCD055.

[0018]

the transformed cell in this invention being prepared by the person skilled in the art with the application of publicly known art, for example, being marketed -- or a person skilled in the art -- general -- acquisition -- it is possible to incorporate DNA of this invention to a suitable host cell using easy various vectors. In that case, it is possible to control a manifestation within the host cell of gene mcd055 arbitrarily by Lycium chinense under the influence of the manifestation regulator gene represented by a promotor and the enhancer in gene mcd055. In production of MCD055 using the transformed host cell, this procedure is used suitably, and also it becomes possible to apply to search of the substance which can adjust research or this gene expression of the manifestation control mechanism of gene mcd055 etc.

[0019]

For example, it can search contacting arbitrary examined substances and the cell transformed by the vector including some or all of gene mcd055 under suitable conditions for the substance which has the operation which promotes or controls the manifestation of gene mcd055 of an examined substance, or it can estimate. DNA which includes a part of mcd055 gene-expression regulatory region, 5' untranslation region, translation initiation site near-field region, or translation field as some examples of gene mcd055 is mentioned.

[0020]

A transgenic animal is producible based on a mouse or other suitable animals combining DNA which is this invention, and a publicly known procedure. If gene mcd055 of this invention is used, it is also possible to produce what is called a knockout animal that destroyed the gene which is equivalent to mcd055 from animals other than Homo sapiens. It becomes possible by pathological [physical / this model animal /, biological, and] and analyzing the genetic feature to solve the function of the gene and protein concerning this invention. It also becomes possible to produce the model animal which has only Homo sapiens mcd055 by introducing Homo sapiens mcd055 of this invention into this animal that is made such and by which the internality gene was destroyed. This model animal is useful to development of the medicine which targeted this Homo sapiens mcd055 introduced, and evaluation.

[0021]

(Protein MCD055)

Protein MCD055 by which a code is carried out to MCD055 is an immunosuppressive receptor which consists of an amino acid sequence shown in SEQ ID NO 2. From the structural aspect looked at by especially the amino acid sequence, it is judged that it is a new immunosuppressive receptor belonging to immunoglobulin superfamily.

[0022]

MCD055 is a molecule which consists of 413 amino acid, and is I type film penetration protein which has a transmembrane domain. From 39 residues to 92 residues of the amino acid sequence shown in SEQ ID NO 2, from 132 residues to 189 residues. The motif GVVYSVV which is missing from the field of 228 to 285 residues, and is conjectured to be functionally equivalent to ITIM by the field of a total of three immunoglobulin domains, 2 to 24 residues, and 317 to 339 residues to a transmembrane domain and the field of 375 to 381 residues exists. Although the range of these fields may produce some difference by the method of a definition of a domain, as long as the same domain is essentially meant, it cannot be overemphasized that it is homonymy.

[0023]
It is judged that protein MCD055 of this invention is an immunosuppressive receptor since it is revealed in the internal organs which are participating in immunity, such as having an immunoglobulin domain in an extracellular domain and having ITIM in an intracellular domain and a spleen, and white corpuscles, directly, and a cell. It is revealed on the cell membrane of an immunocompetent cell, and the immunosuppressive receptor said here refers to the molecule which controls activities, such as cytokine production of the immunocompetent cell, and the proliferation of cells, via phosphorylation control of intracellular activation signal protein, when a ligand, an antibody, etc. combine with an extracellular domain. The antibody to the extracellular domain of the protein of this invention can be produced, and the manifestation in an immunocompetent cell can be checked with a flow cytometer using the antibody. With the system which establishes the T lymphocyte cell strain which made the protein of this invention reveal, gives a cytokine production stimulus to the cell, and measures a production cytokine, It can look for an agony stick antibody [as opposed to / to an index / this invention protein for cytokine production depressant action], or a peptide.
[0024]
The IL-2 production test system using the anti-CD-3 stimulus as a cytokine production stimulus is mentioned as an example. Although intracellular activation signal protein is mentioned [for example, ERK-1 or ERK-2], if it is protein in which phosphorylation is adjusted via MCD055, it will not be limited to these. [that the stimulus by the ligand and agony stick antibody to protein of this invention brings about a control signal in a cell] It can check by phosphorylation control of extracellular signal-regulated kinase-1 (ERK-1) (The Journal of Immunology vol.165 1352-1356 (2000), Carreno et al.).
[0025]
Thus, while MCD055 holds highly the feature observed in an immunosuppressive receptor, such as having an immunoglobulin domain and having ITIM, [055] It has the features, like the manifestation profile characteristic of MCD055 that it is revealed to a lung, liver, the heart, activation white corpuscles, white corpuscles, a spleen, and a trachea as shown in drawing 1 is shown. It is presumed strongly that MCD055 has played from this the characteristic role which is not in other immunosuppressive receptors in regulation of an immune function. Therefore, it is expected that Pharmaceutical Compounds Sub-Division which made MCD055 the target can serve as physic provided with the feature which is not in the former.
[0026]
As long as it is an immunosuppressive receptor, in the amino acid sequence of the protein shown in SEQ ID NO 2, the polypeptide or protein in which one or more amino acid consists of substitution, deletion and/, or added amino acid sequences is also within the limits of this invention.
[0027]
Although the amino acid residue side chains used as a proteinic component differ in hydrophobicity, charge, a size, etc., respectively, some highly preservable relations are known for the meaning of not affecting the three-dimensional structure (it is also called a conformation) of the whole protein substantially. About the substitution of an amino acid residue, for example, a glycine (Gly) and a proline (Pro), Gly, alanine (Ala) or a valine (Val), a leucine (Leu) and isoleucine (Ile), Glutamic acid (Glu), glutamine (Gln) and

aspartic acid (Asp), asparagine (Asn) and cysteine (Cys), threonine (Thr) and Thr, serine (Ser) or Ala, lysine (Lys), arginine (Arg), etc. are mentioned. It is thought that they have the mutually similar character since both Ala, Val, Leu, Ile, Pro, a methionine (Met), a phenylalanine (Phe), a tryptophan (Trp), Gly, and Cys are classified into nonpolar amino acid. As a non-charged polar amino acid, Ser, Thr, a tyrosine (Tyr), Asn, and Gln are mentioned. Asp and Glu are mentioned as an acidic amino acid. Lys, Arg, and a histidine (His) are mentioned as a basic amino acid. Even when spoiling the preservability of an above-mentioned meaning, many variation to a person skilled in the art which, in addition, does not lose the essential function of the protein is also known. The protein of the same kind saved between different living thing kinds focuses or distributes, and, as for deletion or the example which holds the still more essential function even if inserted, many some amino acid is accepted for it.

[0028]

Therefore, if it is an immunosuppressive receptor even if it is the variation protein by substitution on an amino acid sequence shown in SEQ ID NO 2, insertion, deletion, etc., these can be said to be being within the limits of this invention. It being an immunosuppressive receptor will be having a function equivalent to MCD055 protein of this invention, if it puts in another way. Having an equivalent function means holding at least one activity chosen from the phosphorylation inhibiting activities of intracellular activation signal protein, cytokine production inhibiting activities, and cytostatic activity.

[0029]

In a nature, change of such amino acid is accepted like variation produced by gene polymorphism etc., and also it can be artificially performed to a person skilled in the art using a mutagenesis method using mutagens, such as a publicly known procedure, for example, NTG etc., or the site-specific varying method using the various recombination gene procedures. although there is no restriction in particular as long as variation protein of a mutation site and the number of amino acid is an immunosuppressive receptor -- the variation number -- usually -- less than tens of amino acid -- desirable -- less than ten amino acid -- more -- desirable -- 1 -- or it is less than partly.

[0030]

In this invention, he can understand MCD055 as the whole molecule which has all the above domain structure, and also can also understand as partial peptide holding a characteristic domain, especially a domain which bears a binding affinity with a ligand. It is reported for some time that a partial fragment including a ligand binding site is separated from others and a domain, with a characteristic conformation held, and may exist as partial peptide (or called solubilization and a meltable type) of isolation in some film penetration type protein. Since such partial peptide holds a binding affinity with a still specific ligand, search of a compound which has a binding affinity to this protein using this of it is attained. As long as it has the ligand binding ability in this meaning, if partial peptide in MCD055 is also a substance equivalent to this invention substantially, he should understand it. An immunoreaction is adjusted by competition with MCD055 (film penetration type on a cell membrane), or meltable type MCD055 the very thing functions as a ligand, meltable type MCD055 is combined with a certain receptor, and a possibility of demonstrating activity which adjusts an immunoreaction is also considered. Partial peptide which has such activity is also a thing of this invention within the limits. Activity which adjusts an immunoreaction of meltable type MCD055 can be detected by

a mixed lymphocyte reaction shown in Embodiment 9. Activities which adjust an immunoreaction are specifically cytokine production regulation activity and/or proliferation-of-cells regulation activity.

[0031]

As a desirable mode of partial peptide, the peptide which includes either an extracellular domain (25-316a.a. of SEQ ID NO 2) or an intracellular domain (340-413a.a. of SEQ ID NO 2), for example is mentioned. Existence of a transmembrane domain is predicted 339th near [the 317th to] the amino acid residue of the amino acid sequence of SEQ ID NO 2 (when film penetration prediction program SOSUI is used). Such a difference should be permitted although by the amino acid residue to where a domain is divided may get mixed up somewhat with the prediction method of the domain to be used. It is preferred to include an immunoglobulin domain at least in the viewpoint of having the activity which adjusts ligand binding ability or an immunoreaction. It is preferred that ITIM of an intracellular domain is included at least in the viewpoint of having a signaling function. As long as at least one functional domain is included, all or some of other domains may connect, and it may become other protein and a fusion protein with a peptide.

[0032]

A functional domain is partial peptide holding the activity or the signaling function which adjusts ligand binding ability and an immunoreaction. As long as a fusion protein has at least one activity chosen from the activity or the signaling function which adjusts the ligand binding ability as MCD055 or meltable type MCD055, and an immunoreaction, there is no restriction in particular in other polypeptides connected with MCD055 partial peptide. Although desirable examples of such a fusion protein are a fusion protein with the Fc fragment of an immunoglobulin, and a fusion protein with a histidine tag, they are not limited to these. In the case of IgG, an Fc fragment consists of a hinge region, CH2 field, and CH3 field. It is usable also in the portion (for example, independent or arbitrary combination of each field of a hinge region, CH2 field, CH3 field, or CH4 field) of an Fc fragment. Although any may be sufficient as the kind in which the immunoglobulin in this case originates, the thing of the Homo sapiens origin is preferred. moreover -- necessarily not being limited about a class and a subclass -- both IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 IgM IgE IgD, etc. -- although -- although it is usable, IgG is a desirable example, for example.

[0033]

In the protein which has a signal sequence, that from which the signal sequence was cut may be functioning as a maturation protein. Therefore, if the mature peptide in which the signal sequence was removed from the protein of this invention is also a substance equivalent to this invention substantially, he should understand it. In the case of MCD055, existence of a signal sequence is predicted 24th near [the 2nd to] the amino acid residue of the amino acid sequence shown in SEQ ID NO 2 (when film penetration prediction program SOSUI is used). Although the range of a signal peptide field may produce some difference by a prediction program and diversity may arise in how a signal peptide is removed also in the protein produced experimentally, As long as it is functionally equivalent as MCD055 protein, it should be understood that it is a substance equivalent to this invention.

[0034]

The protein of this invention or its partial peptide is applicable to search of the substance which adjusts the activity of this protein. It is expected that compounds obtained through search will serve as the effective therapeutic drug or prophylactic to the disease to which the protein of this invention relates, for example, an autoimmune disease, immune disorder, an allergic disease, an inflammatory disease, a tumor, etc.

[0035]

(Antibody)

This invention provides the antibody further combined with MCD055. The antibody of this invention is an antibody which recognizes the MCD055 whole or its partial peptide specifically as an antigen, and a monoclonal antibody and/or a polyclonal antibody are contained. It may belong to any of five classes (IgG, IgA, IgM, IgD, IgE) classified as the structure, physicochemical quality, and immunological property of an immunoglobulin, or the subclass by the type of H chain. It may be fragments, such as Fab when $F(ab')_2$ when pepsin decomposes, and a papain decompose an immunoglobulin, or they may be a chimeric antibody and a hominization antibody. The antibody which it not only recognizes MCD055 or its partial peptide specifically, but has the function to adjust the activity of MCD055 is also contained in this invention. With the antibody which has the function to adjust the activity of MCD055, the neutralizing antibody which checks combination of MCD055 and a ligand, for example is mentioned. These antibodies are useful to detection [be / research / or / it / clinical] of MCD055, etc.

[0036]

(Antisense nucleic acid)

This invention provides what is called antisense nucleic acid that can control MCD055 biosynthesis in a nucleic acid level in in the living body. The transfer stage from a genom region required for this antisense nucleic acid to make mRNA which encodes MCD055 to pre-mRNA, . [the processing stage from pre-mRNA to mature mRNA, nuclear membrane passage stage, or translate phase to protein] It may combine with DNA or RNA which bears gene information, what affects the normal flow of transfer of genetic information and adjusts a proteinic manifestation may be meant, and it may consist of arrangement which has complementation into the whole nucleic acid sequence of gene mcd055, or one of portions. It is the nucleic acid (DNA and RNA are included) which comprises the arrangement which is equivalent to a nucleic acid sequence given in SEQ ID NO 1 or SEQ ID NO 3, or has complementation preferably. When it is a form where mRNA transferred from a genom region includes an untranslation region in intron structure or a five prime end, or a three-dash terminal, I will have the considerable or function that the antisense nucleic acid which has complementation is also equivalent to the antisense nucleic acid of this invention, in the arrangement of this non-translating portion.

[0037]

The antisense nucleic acid of this invention contains all the various inductors with which DNA, its conformation besides RNA, and a function are similar with DNA or RNA. For example, the nucleic acid which other substances combined with the three-dash terminal or the five prime end, the base of an oligonucleotide, The nucleic acid which has the base, sugar, or phosphoric acid which does not exist, the nucleic acid which has frames (backbone) other than a sugar-phosphoric acid frame, etc. are mentioned to sugar, the nucleic acid which substitution and a modification produced in any at least one of the

phosphoric acid, and nature. These nucleic acid is preferred as an inductor in which at least one of nuclease tolerance, tissue selectivity, cell permeability, and the avidity was raised. That is, as long as it has a function which can control the activity manifestation of MCD055, there is no restriction in the form of nucleic acid.

[0038]

The antisense nucleic acid which has a complementary base sequence in a base sequence which is generally hybridized in this invention into the loop portion of mRNA which forms the stem loop, i.e., the base sequence of the field which forms the stem loop, is preferred. Or antisense nucleic acid which is combined with near a translation initiation codon, a ribosome binding site, a capping site, and a splice site, i.e., the antisense nucleic acid which has complementary arrangement in the arrangement of these parts, is preferred at the point that generally high manifestation depressor effect is expectable.

[0039]

In order to make such antisense nucleic acid take in in a cell and to make it act efficiently, 30 or less base 15 or more-base of things which consist of 15 or more base a base sequence which consists of the 18 or more base number of bases of 22 or less base more preferably 25 or less base are preferably preferred for the chain length of the antisense nucleic acid of this invention.

[0040]

[the manifestation depressor effect of the antisense nucleic acid of this invention] A publicly known procedure, for example, the gene expression regulatory region of this invention, 5' untranslation region, The manifestation plasmid which connected reporter genes, such as a luciferase, with DNA including a part of translation initiation site near-field region or translation field is produced, in . [vitro transcription reactions (Promega: Ribo max system etc.) and in vitro translation reactions (Promega: Rabbit Reticulocyte Lysate System etc.)] It can evaluate, when the gene of this invention like the system used together adds an examined substance in a system under the environment transferred or translated and measures the amount of manifestations of this reporter gene.

[0041]

Since the antisense nucleic acid of this invention can control the manifestation of MCD055 in the living body, it is useful as prevention and a treating agent of the disease to which MCD055 relates.

[0042]

[Mode for carrying out the invention]

(Nucleic acid)

[DNA of this invention] [as an example acquired from a DNA library] A suitable genomic DNA library and cDNA library are screened with the screening procedure by hybridization, the immuno screening procedure using an antibody, etc., the clone which has target DNA is proliferated, and there is the procedure of starting using a restriction enzyme etc. from there. [screening by a hybridization method] Carry out the label of the DNA which has a base sequence of a description, or its part to SEQ ID NO 1 by ^{32}P etc., consider it as a probe, and arbitrary cDNA libraries are received, It can carry out by a publicly known procedure (for example, Molecular Cloning, such as Maniatis T., a Laboratory Manual, ColdSpring harbor Laboratory, New York, 1982). The antibody of this invention mentioned later can be used for the antibody used with an immuno screening procedure. New DNA of this invention can be obtained also by PCR

(Polymerase Chain Reaction) which uses a genomic DNA library or a cDNA library as a mold. PCR based on the base sequence of a description to SEQ ID NO 1 A sense primer, Produce an antisense primer and arbitrary DNA libraries are received, A publicly known procedure (for example, refer for Michael A.I. etc. PCR Protocols, a Guide to Methods and Applications, Academic Press, and 1990) etc. can be performed, and DNA of this invention can also be obtained. The DNA library which has DNA of this invention is chosen and used for the DNA library used by the various above-mentioned procedures. If the DNA library concerned is a library which has DNA of this invention, [the library] It is usable, and anythings use a commercial DNA library, or, A cell suitable for producing a cDNA library is chosen from the cell which has DNA of this invention, [in accordance with a publicly known procedure (J. refer to it Molecular Cloning, such as Sambrook, aLaboratory Manual 2nd ed., Cold Spring Harbor Laboratory, New York, and 1989)] A cDNA library can be produced and used.

[0043]

It is also possible to prepare with the chemical synthesis procedures, such as a phospho aminodite method, based on the arrangement indicated by this Description.

[0044]

The recombination vector containing DNA of this invention may be a thing of what kind of forms, such as annular and straight chain shape. As long as DNA of this invention is required for this recombination vector in addition all or in part, it may have other base sequences. A part is DNA which encodes partial peptide of the protein of this invention. With other base sequences, the arrangement of an enhancer, a promotor's arrangement, a ribosome junction sequence, They are things, such as a base sequence etc. of the gene used as the base sequence used for the purpose of amplification of a copy number, the base sequence which encodes a signal peptide, the base sequence which encodes other polypeptides, poly A addition arrangement, splicing arrangement, a replicator, and a selective marker. A desirable example of the recombination vector of this invention is an expression vector.

[0045]

It is also possible to add a translation initiation codon and a translation stop codon to DNA of this invention using a suitable synthetic DNA adapter, or to make it newly generate or to vanish restriction enzyme cutting arrangement suitable in a base sequence when modifying a gene. These are within the limits of the work which a person skilled in the art usually does, and can be processed arbitrarily and easily based on DNA of this invention.

[0046]

The vector holding DNA of this invention should just use it for the suitable vector according to the host who uses it, choosing, it is also possible to use various viruses other than a plasmid, such as a bacteriophage, a baculovirus, a retrovirus, and a vaccinia virus, and there is no restriction in particular.

[0047]

The gene expression of this invention can be made to reveal under control of promotor arrangement characteristic of this gene. If this manifestation system is used, search of the substance promoted or controlled can perform transfer of the gene of this invention more advantageously. Or another, suitable manifestation promotor in the upper stream of the gene of this invention can also be used for promotor arrangement characteristic of this

gene, connecting or replacing. In this case, the promotor who uses it should just choose suitably according to a host and the purpose of a manifestation, for example, when a host is coliform bacillus, [T7 promotor, a lac promotor, a trp promotor, lambdaPL promotor, etc.] When a host is yeast, PHO5 promotor, a GAP promotor, An ADH promotor etc. can illustrate an SV40 origin promotor, a retroviral promotor, an elongation factor 1 alpha (Elongation Factor 1alpha) promotor, etc., when a host is an animal cell, but though natural, it is not limited to these.

[0048]

The procedure of introducing DNA into a vector is publicly known (J.). [Sambrook etc.] Molecular Cloning, a Laboratory Manual 2nd ed., Cold Spring Harbor Laboratory, New-York (New York), 1989, reference. That is, what is necessary is just to carry out ligation of each fragment obtained by digesting DNA and a vector with a respectively suitable restriction enzyme using a DNA ligase.

[0049]

(Protein)

The protein of this invention can be prepared from the cell which has revealed this protein, or tissue, It can prepare also by the procedure of rearranging using the suitable host cell chosen from a procaryote or an eukaryotic organism also by the chemical synthesis method which uses a peptide synthesis machine (for example, a peptide synthesizer 433A type, the Applied Biosystems Japan, Ltd. make). However, the production and recombinant protein by the genetic engineering procedure are preferred from the field of the purity.

[0050]

There is no restriction in particular in the host cell made to transform using the recombination vector of the preceding clause, E. Many cells, such as an animal cell represented by an available lower cell, an insect cell, COS7 cell, a CHO cell, and HeLa cells in the genetic engineering technique represented by coli, B.subtilis, or S.cerevisiae, are available also to this invention.

[0051]

The transformed cell of this invention can be obtained by making a suitable host cell transform by the recombination vector of this invention. [as a procedure of introducing the recombination vector of the preceding clause into a host cell] The electroporation method, the protoplast method, an alkali metal method, the calcium phosphate precipitating method, Which procedure may be used although there are the publicly known procedures (an experimental-medicine special issue, genetic engineering handbook March 20, 1991 issue, Yodosha, reference), such as the DEAE dextran process, a microinjection method, and a method of using virion.

[0052]

In order to produce the protein concerned in genetic engineering, an above-mentioned transformed cell is cultured, cultivation mixtures are collected, and the protein concerned is refined. Cultivation of a transformed cell can be performed by a general procedure. Since there are various kinds of compendiums (the volume examples and for "microorganism laboratory procedure" Japanese Biochemical Society, Tokyo Kagaku Dojin Co., Ltd., 1992) about cultivation of a transformed cell, they can be referred to and can be performed.

[0053]

As a procedure of refining the protein of this invention, out of the procedure usually used for refining of protein, a suitable procedure can be chosen suitably and can be performed from a cultivation mixture. Namely, a salting-out method, ultrafiltration, the isoelectric point precipitating method, gel filtration technique, electrophoresis, Various affinity chromatography, such as an ion exchange chromatography, a hydrophobic chromatography, and antibody chromatography, What is necessary is to choose a suitable procedure suitably out of procedures which may usually be used, such as the chromatofocusing method, an adsorption chromatography, and reversed phase chromatography, and just to refine in a suitable order as occasion demands using an HPLC system etc.

[0054]

It is possible to also make the protein of this invention reveal as other protein or a fusion protein with a tag (an example, a glutathione S transferase, protein A, a hexa histidine tag, a FLAG tag, others). The united type made to reveal becomes it is possible to start using a suitable protease (an example, a thrombin, ENTERO kinase, others), and possible [sometimes preparing protein more advantageously]. When what is necessary is just to have performed refining of the protein of this invention, combining suitably a procedure general to a person skilled in the art and it is made revealed with especially the form of a fusion protein, it is preferred to adopt a purification method characteristic of the form.

[0055]

It is one of the procedures which also produces the procedure of acquiring using a recombinant DNA molecule with the synthesizing method (J. Sambrook, etal.:Molecular Cloning 2nd ed. (1989)) of a cell free system in genetic engineering.

[0056]

Thus, the protein of this invention can be prepared with its independent form or the form of a fusion protein with the protein of another kind, and these things [it not being restricted to seeing and transforming the protein of the invention in this application to further various forms] are also possible. For example, processings by the various procedures known by the person skilled in the art, such as combination with polymers, such as various chemical modification to protein and a polyethylene glycol, and combination to an insoluble carrier, can be considered. the existence of addition of a sugar chain by the host who uses -- or -- to that extent -- being also alike -- a difference is accepted. Even if it is in this case, as long as it functions as an immunosuppressive receptor, in addition, it should be said that it is under the thought of this invention.

[0057]

The protein of this invention is applicable to screening of the substance which is used as an antigen for producing an antibody, or is combined with this protein, or the substance which adjusts the activity of this protein, and useful.

[0058]

As for MCD055 of this invention, it is possible by culturing the above transformed cells, especially an animal cell to make the cell surface high-reveal the purpose molecule. In manufacturing suitable fragments, such as an extracellular region protein fragment of MCD055, as soluble protein, on the other hand, It can manufacture by preparing a transformed cell as mentioned above using DNA which encodes the extracellular region concerned or each domain, and making it secrete in a culture supernatant by culturing an ectoplasm conversion cell.

[0059]
When MCD055 exists in the periplasm of a transformed cell, or cytoplasm, on the other hand, To the cell suspended to suitable buffer solution, for example, sonication, a freezing and thawing method, Or after performing lysozyme treatment etc. and destroying a cell wall and/or a cell membrane, the membrane fraction which contains the protein of this invention by procedures, such as centrifugal separation and filtration, is obtained, this membrane fraction is further solubilized using a suitable surface-active agent, and rough solution is prepared. and the law from the rough solution concerned -- by a method, it can isolate and the purpose protein can be refined.
[0060]
(mcd055 gene-recombination nonhuman animal)
This invention provides a mcd055 gene-recombination nonhuman animal. A transgenic nonhuman animal and a KO nonhuman animal are contained in a mcd055 gene-recombination nonhuman animal. When a mcd055 gene-recombination nonhuman animal incorporates artificially the gene which encodes the protein of this invention on the chromosome of this animal, the grade of a manifestation of the protein of this invention, a manifestation stage, a manifestation part, etc. are controlled. As a nonhuman animal, although a cow, a sheep, a goat, a swine, a mouse, a horse, a fowl, etc. are mentioned, for example, it is not limited to these. A nonhuman mammal is preferred also in a nonhuman animal.
[0061]
If gene mcd055 of this invention is used, a transgenic nonhuman animal is producible. a law for which this transgenic nonhuman animal is usually used in manufacture of a transgenic animal -- in accordance with a method (the volume an example, a developmental engineering experiment manual, Kodansha SAIENTIFIKU issue, and for Motonari Katsuki, Tatsuji Nomura editorial supervision, 1987), it is producible. That is, the gene or recombination vector of this invention is introduced into the fertilized egg of a nonhuman animal, this fertilized egg is generated to an individual, and the individual by which transgene was incorporated into the genome of a somatic cell is sorted out.
[0062]
Specifically, in the case of a transgenic mouse, DNA built so that mcd055 gene could be revealed to the pronucleus term fertilized egg acquired from normal C57Black/6 mouse is poured in directly, for example. The construct which connected and introduced mcd055 gene downstream from the suitable promotor is more specifically produced, straight-chain-shape DNA which removed the arrangement of procaryote origin as much as possible when it was necessity after that is obtained, and this is directly poured into a pronucleus term fertilized egg pronucleus using a detailed glass needle.
[0063]
This fertilized egg is transplanted to the uterus of another false pregnancy mouse used as assumed parents. An ICR female mouse is crossed with the male mouse which cut or litigated the spermatic duct, and a false pregnancy mouse generally produces it. From the organization of ** of transplant ******, genomic DNA is extracted, the existence of introduction of MCD055 gene is checked by the PCR method or the Southern-blotting method, and a transgenic mouse is obtained.
[0064]
Based on the base sequence of mcd055 (or mouse homologous gene of mcd055), what is

called a "knockout mouse" is producible. [in this invention / the "knockout mouse"] It is the mouse in which the internality gene which encodes the protein of the mouse derived of this invention was knocked out (inactivation), for example, the positive negative selection method (the US,5,464,764,B gazette.) adapting homologous recombination Are producible using the No. 5,487,992 gazette, the No. 5,627,059 gazette, Proc.Natl.Acad.Sci.USA, Vol.86-8932-8935-1989, Nature, Vol.342,435-438-1989, etc., Such a knockout mouse is also one mode of this invention. The destructive animal of this gene may be obtained as a result also by the gene disruption procedure of the random animal individual performed briskly in recent years. The animal it became clear destroying of this gene is one mode of this invention, when it is not concerned with whether for the destruction to have had the purpose with this clear gene, and to have been performed but destruction of the gene becomes clear.

[0065]

Or also in inside and a large animal, creation of a cloned animal by a nuclear transplantation was attained recently. In connection with this, creation of TRANS GENIC using this art and a knockout animal also actually came to be performed. That is, a core can be obtained from a cell obtained by performing homologous recombination the same with carrying out to an embryonic stem cell based on a base sequence of mcd055 (or homologous gene of mcd055 in each animal) to a somatic cell or a cell of a germ cell line, and a cloned animal can be obtained using the core. This animal is a knockout animal in which mcd055 (or homologous gene of mcd055 in each animal) was lost. Or it is also possible by introducing mcd055 (or homologous gene of mcd055 in each animal) gene into arbitrary cells of arbitrary animals, and obtaining a cloned animal like an above-mentioned procedure, using the core to produce a transgenic animal. Such a KO nonhuman animal and a transgenic nonhuman animal are not concerned with the kind, but are one mode of this invention.

[0066]

(Antibody)

Both a polyclonal antibody and a monoclonal antibody can obtain an antibody of this invention by the ability to refer to a publicly known procedure (for example, the immunity experiment operating method, edited by Japanese Society for Immunology, the Japanese Society for Immunology issue, reference). It explains briefly [below].

[0067]

In order to obtain the new antibody concerned, if the protein of this invention is inoculated into an animal if needed with suitable adjuvant, such as Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA), as immunogen and it has necessity first, booster immunization will be carried out at intervals of two to four weeks. Blood collecting is performed after booster immunization and an antiserum is obtained. The protein of this invention used as an antigen may be obtained by what kind of procedure, as long as it is a thing of the degree of refining which can be used for production of an antibody. The partial polypeptide of the protein of this invention can also be conveniently used as immunogen. What is necessary is to combine it with carriers, such as keyhole limpet hemocyanin (KLH), and just to use it as an antigen, when the polypeptide used as immunogen is the polypeptide of a low molecule, i.e., the polypeptide which consists of about ten to 20 amino acid. Although what kind of thing the animal which carries out immunity may be, it is preferred to desirable usually use it

by a person skilled in the art from the rat used for an immunological experiment, a mouse, a rabbit, a sheep, a horse, a fowl, a goat, a swine, a cow, etc., choosing the animal species which can produce the target antibody.
[0068]
A polyclonal antibody can be obtained by refining the obtained antiserum. What is necessary is just to perform refining, combining suitably the publicly known procedures, such as salting out, an ion exchange chromatography, and affinity chromatography.
[0069]
For obtaining a monoclonal antibody, it carries out as follows. That is, antibody producing cells, such as splenic cells or a lymphocyte, are extracted from the animal which carried out immunity, by the publicly known procedure of using a polyethylene glycol, Sendai Virus, an electric pulse, etc., it unites with a myeloma cell strain etc. and a hybridoma is produced. Then, what is necessary is to choose and cultivate the clone which is producing the antibody combined with the protein of this invention, and just to obtain by refining the culture supernatant of the selected clone. What is necessary is just to use refining, combining suitably the publicly known procedures, such as salting out, an ion exchange chromatography, and affinity chromatography.
[0070]
Even if it uses the genetic engineering procedure, the new antibody concerned is obtained. For example, mRNA is extracted from the hybridoma which produces the monoclonal antibody to the splenic cells, the lymphocyte, this invention protein, or its partial polypeptide of the animal which carried out immunity by this invention protein or its partial polypeptide, and a cDNA library is produced based on this. The clone obtained by screening the clone which is producing the antibody reacted to an antigen can be cultivated, and the target antibody can be refined combining the publicly known procedure from a cultivation mixture. When using an antibody for treatment, the point of immunogenicity to a hominization antibody is preferred. A hominization antibody can be prepared by carrying out the immunity of the mouse (example Nat. Genet. 15;146-156 (1997)) which replaced the immune system with the human thing. It can also prepare in genetic engineering using the hypervariable region of a monoclonal antibody (Method in Enzymology 203; 99-121 (1991)).
[0071]
(Antisense nucleic acid)
Antisense nucleic acid can be manufactured by the publicly known procedure (for example, the volume Stanley T.Crooke and on Bernald Lebleu, in Antisense Research and Applications, CRC publication, Florida, 1993). If it is natural DNA and RNA, it can compound using a chemical synthesis machine, or the antisense nucleic acid of this invention can be obtained by the PCR method by using MCD055 as a mold. In an inductor, a thing compoundable using a chemical synthesis machine (for example, made in applied bio-systems Japan, Inc., Expedite Model 8909) also has a methyl phosphonate type, a phosphorothioate type, etc. In this case, antisense nucleic acid can be obtained also by refining the synthetic product acquired by operating it according to the manual attached to the chemical synthesis machine by the HPLC method using reversed phase chromatography etc.
[0072]
In using DNA and antisense nucleic acid of this invention as a probe for diagnosis, in

accordance with a publicly known procedure, it carries out the sign of them with radioactive isotope, enzyme, a fluorescent substance, or scintillating material. Next, DNA or mRNA is prepared by the publicly known procedure from a specimen, by making this into an examined substance, after adding said sign probe and making it react, it washes and said unreacted sign probe is removed. If gene mcd055 or RNA is contained in the examined substance, the antisense nucleic acid concerned will be combined with them. The existence of bonding can know as an index luminescence by enzyme, a fluorescent substance, scintillating material, or radioisotope etc. which carried out the sign, fluorescence, radioactivity, etc.

[0073]

When using DNA, the antisense nucleic acid, or the recombination vector of this invention for a physic use, it is preferred to use it with the directions for use which are permitted pharmacologically and deal in the thing of purity suitable for using it as a drug.

[0074]

DNA, the antisense nucleic acid, or the recombination vector of this invention may use them for a directly suitable solvent, being dissolved or suspended, and it may be used, making it into the form which was enclosed in the liposome or was included in the suitable vector. It may be used for suitable drug designs, such as the injection, a tablet, the capsule, ophthalmic solution, cream pharmaceuticals, a seat agent, nebula, and poultice, adding the auxiliary ingredients which may be permitted pharmacologically if needed, and carrying out. The auxiliary ingredients which may be permitted pharmacologically are things, such as a solvent, a base, a stabilizing agent, a preservative, a solubilizer, an excipient, and a buffer.

[0075]

DNA, the antisense nucleic acid, or the recombination vector of this invention can set up and use the medication method and its dose according to a patient's age, sex and the kind of disease, and a grade, when it is considered as the above drug designs. Namely, what is necessary is to choose a suitable procedure from internal use or inhalation, dermal administration, instillation, intravaginal medication, intra-articular medication, rectum medication, intravenous administration, local administration, intramuscular administration, hypodermic administration, intraperitoneal injection, etc., and just to prescribe a quantity suitable for improving symptoms for the patient.

[0076]

(Screening method)

The transformed cell in which this invention has revealed the protein of this invention, and this protein, It is characterized by using the transformed cell or mcd055 gene-recombination nonhuman animal transformed by the recombination vector containing DNA of this invention, and this DNA, and this recombination vector, and is related with the procedure of screening the substance which adjusts the function or manifestation of the protein of this invention. Prepare (1) examined substance and this examined substance is more specifically contacted to the transformed cell which has revealed the protein or this protein of this invention, The procedure (2) examined substance in which this examined substance consists of evaluating whether it has the operation which adjusts the activity of the protein of this invention is prepared, This examined substance is contacted to the transformed cell transformed by the recombination vector or this recombination vector containing DNA of this invention, and the procedure this examined

substance consists of evaluating whether it has the operation which adjusts mcd055 gene expression etc. are mentioned. As an example of (1), in the system shown in Embodiment 7, 8, or 9, the activity of the protein of this invention under the bottom of examined substance existence / nonexistence is measured, and the procedure of choosing the examined substance which increases or decreases the activity of the protein of this invention in the bottom of existence compared with the bottom of nonexistence is mentioned. As an example of (2), mcd055 gene-expression regulatory region, 5' untranslation region, The recombination vector which connected reporter genes, such as a luciferase, with DNA including a part of translation initiation site near-field region or translation field is produced, The amount of manifestations of this reporter gene is measured under the bottom of existence of an examined substance / nonexistence under the environment where the gene of this invention is transferred or translated, and the method of checking the transfer promotion activity or transfer inhibiting activities of an examined substance is mentioned. The transformed cell in which the screening method of this invention has revealed the protein of this invention, and this protein, The recombination vector containing DNA of this invention, and this DNA, The process at which an examined substance is contacted with the transformed cell or mcd055 gene-recombination nonhuman animal transformed by this recombination vector;. [whether the activity of the protein of this invention in an examined substance addition group and an examined substance additive-free group or the expression level of DNA of this invention has a difference] The process to detect; the process of choosing the examined substance in which the difference was accepted as the activity pacemaker of the protein of this invention or an expression control substance of DNA of this invention may be included. Any of a substance which have the operation (antagonist) which copies thru/or (mimic) enhances the activity of MCD055 protein (agonist), or checks it may be sufficient as the substance in which the operation which adjusts the activity of the protein of this invention is shown. Any of a substance which have the operation which promotes or controls the manifestation of gene mcd055 may be sufficient as the substance in which an expression control operation of DNA of this invention is shown. . [whether an examined substance shows the activity accommodation of the protein of this invention, or an expression control operation of DNA of this invention] What is necessary is just to investigate whether the activity of the protein the case where an examined substance is added in the system which can check the manifestation of the system or DNA which can check proteinic activity, and in additive-free, or the expression level of DNA has a difference. With the expression level of DNA, any of the manifestation strength of mRNA of mcd055 gene and proteinic manifestation strength may detect. The expression level of a reporter gene may be detected not as the expression level of mcd055 gene or the MCD055 protein itself but as substitution. A reporter assay system says the assay system which screens the substance which acts on this manifestation regulatory region by making into an index the amount of manifestations of the reporter gene arranged downstream from gene expression regulatory region to examine. As manifestation regulatory region, a promotor, an enhancer, the CAAT box usually seen in a promoter region, TATA box, etc. can be illustrated. As a reporter gene, a CAT (chloramphenicol acetyltransferase) gene, a luciferase (luciferase) gene, a beta galactosidase (beta-galactosidase) gene, etc. can be used. The gene expression regulatory region and 5' untranslation region of this invention can be acquired by a publicly known procedure (a

new cell technology experiment protocol (Shujunsha), 1993). Having the operation which has or enhances the operation to check (or control) (or promotion) means that a difference has the measured value of proteinic activity or the expression level of DNA between an examined substance addition group and an examined substance additive-free group. For example, the prevention (or control) rate or reinforcement (or promotion) rate calculated by the following formula says more preferably that it is not less than 90% preferably especially not less than 70% still more preferably not less than 50% not less than 30% not less than 10%.

[0077]

Measured value x100 of the absolute value / additive-free group of prevention (or control) rate or reinforcement (or promotion) rate = (measured value of a measured value-examined substance addition group of an examined substance additive-free group)

[0078]

Here, about which activity of prevention or reinforcement it is, and measured value, it is suitably set by a kind of system which can check a manifestation of a system or DNA which can check proteinic activity. For example, when a system which can check proteinic activity is a system of measurement of cytokine production inhibiting activities. When the amount of cytokine production can be used as measured value and it becomes the amount of cytokine production of an amount of cytokine production > examined substance additive-free group of an examined substance addition group, it can be said that there is MCD055 protein activity inhibitory action in an examined substance. When a value of a background or a noise is included in a system of measurement, it cannot be overemphasized that a value which deducted such a thing is made into measured value.

[0079]

[compounds obtained from protein which is this invention being an immunosuppressive receptor through search using an above-mentioned screening method or a transgenic animal] Becoming an effective therapeutic drug or a prophylactic to an autoimmune disease, immune disorder, an allergic disease, an inflammatory disease, etc. is expected. Although protein, a peptide, an oligonucleotide, a synthetic compound, a natural origin compound, a fermentation product, cell extract, vegetable extract, animal tissue extract, etc. are mentioned as an examined substance, it may not be limited to this, but a new substance or a publicly known substance may be sufficient.

[0080]

[Working example]

Although this invention is further explained in full detail according to the following embodiments, he limits this invention to these embodiments, and it should not be understood.

[0081]

Embodiment 1 Cloning of gene mcd055

(1) Acquisition of clone C-SPLEN2010588

(1-1) Production of a cDNA library, and evaluation of full length nature

mRNA extracted as all the RNA from the spleen (Spleen) of human tissue was purchased in the state of all the RNA (CLONTECH #64034-1). It is oligo KYAPU method [M. Maruyama and S. Sugano, Gene, and 138 from purchased RNA. : The cDNA library was produced by the procedure (WO 01/04286) which improved 171-174] (1994). Oligo-cap linker and Oligo dT primer are used, As indicated to WO 01/04286, BAP (Bacterial

Alkaline Phosphatase) processing, Synthesis of TAP (Tobacco Acid Pyrophosphatase) processing, RNA ligation, and the first chain cDNA and removal of RNA were performed. Subsequently, it changed into double-strand cDNA by PCR (polymerase chain reaction) using the PCR primer by the side of 5 'a side and 3', and cut by SfiI. Subsequently, cloning of the directivity of cDNA was decided and carried out to vector pME18SFL3 (GenBank AB009864, Expression vector) which cut the cDNA fragment which carried out fractionation to 2 or more kbs by DralII, and the cDNA library was produced. The arrangement of the PCR primer by the side of Oligo-cap linker, Oligo dT primer, and 5 'a side and 3' used what was indicated to WO 01/04286.

[0082]

[plasmid DNA / of the clone obtained from these] A DNA sequencing reagent (BigDye Terminator Cycle Sequencing FS Ready Reaction Kit, product made by PE Biosystems) is used for the base sequence of 5' end of cDNA, According to the manual, the DNA sequence was analyzed after the sequencing reaction by the DNA sequencer (ABI PRISM 3700, product made by PE Biosystems). The obtained data was put in a database.

[0083]

It asked for the rate of full length of the 5'-end of about 30,000 clones of the cDNA library of the Homo sapiens spleen (Spleen) produced by the procedure which improved Oligo-capping method by the following procedure. [clones / whose Homo sapiens known mRNA in a public database and 5'-terminal sequences correspond / all the] Although the 5'5when - end is extended'-end was short for a long time than the known mRNA arrangement in a public database, the translation initiation codon judged the case where it had to be "full length", and judged the case where the translation initiation codon was not included to be "non-full length." Rate [of a 5'-end] of full length [number of full length clones/(number of number of full length clones + non-full length clones)] was calculated based on this. As a result, the rate of full length of the 5'-end was about 90%. It turned out that the rate of full length of the 5'-end arrangement of the clone from the acquired cDNA library is dramatically higher than this result.

[0084]

(1-2) Selection and full length sequence analysis of a full length base sequence analysis clone

About the five prime end arrangement of the cDNA clone produced by performing it above, homogeny search by GenBank and BLAST for data with the notation of complete cds of UniGene was performed, and the same thing removed in human mRNA arrangement. Next, it clusters, and when not less than 90% of homogeny and a consensus sequence were 50 or more base pairing, it was regarded as the same group and the group was made to form. The longer clone to a 5'- side in a group was chosen, and the clone which conducts full length base sequence analysis was obtained.

[0085]

The full length's cDNA base sequence was determined by the usual procedure about the clone selected by full length base sequence analysis. The full length base sequence was decided so that a partial base sequence might overlap both chains completely. C-SPLEN2010588 was acquired from the inside of it.

[0086]

[clone C-SPLEN2010588] . Encode new protein MCD055 which consists of 413 amino acid denoted by SEQ ID NO 2. cDNA which consists of a base sequence (SEQ ID NO 3)

of full length 1997 base pairing containing the open reading frame (ORF) which consists of a base sequence of 1239 bases denoted by SEQ ID NO 1 was contained. This plasmid C-SPLEN2010588 is deposited with International Organism Depository, NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY, which is an international microorganism depository institution as accession number FERM P-19165.

[0087]

(2) Analysis of a cDNA clone base sequence and an amino acid sequence

(2-1). [MCD055 amino acid sequence] a HMMER program (R. --) [Durbin and] S. By Eddy, A. Krogh, G. Mitchison. Cambridge University Press, and . (1998). [a Pfam database (http://www.sanger.ac.uk/Pfam/)] When searched, it applied to 285 residues from 92 residues, 189 residues from 132 residues, and 228 residues from 39 residue, and a total of three immunoglobulin domains were seen.

[0088]

(2-2) Film penetration prediction program (Takatsugu.) Hirokawa and Seah Boon-Chieng. and Shigeki Mitaku, SOSUI: Classification. and Secondary Structure. By Prediction System for Membrane Proteins and Bioinformatics (formerly CABIOS) 1998 May;14(4):378-379. [film penetration part prediction] The transmembrane domain was predicted by 2 to 24 residues, and 317 to 339 residues in the amino acid sequence shown by SEQ ID NO 2 as a result of carrying out. In the amino acid sequence shown by SEQ ID NO 2 from this, from the 2nd Leu to the 24th Thr was predicted to be signal peptide arrangement. Since other transmembrane domains were one by the side of a C terminus, it was predicted that this protein exists in the cell membrane surface in the form where the N terminus side was turned out of the cell, and it turned the C-terminus side in the cell.

[0089]

(2-3) Although [IV]xYxx[LV] is generally known as an ITIM motif (J Immunol. Vol.159(5):2075-7 (1997)), It accepted [which is conjectured to be functionally equivalent to this motif] to 375 to 381 residues of the amino acid sequence shown by SEQ ID NO 2, having covered it GVVYSVV.

[0090]

The check of the manifestation profile by embodiment 2 PCR

Organization manifestation profile analysis of mcd055 by RT-PCR was conducted. The Homo sapiens RNA used for analysis is as follows. the law from the Homo sapiens coronary-arteries endothelial cell (HCAEC) and the Homo sapiens coronary-arteries vascular smooth muscle cells (CASMC) -- mRNA was prepared by the method. after cultivating Homo sapiens peripheral blood origin white corpuscles under existence of 50 microg/ml PHA or nonexistence for 24 hours -- mRNA -- a law -- it prepared by the method. mRNA of a human lung, the kidney, the pancreas, a spleen, the heart, the suprarenal gland, a testis, the trachea, the embryo kidney, and the brain was purchased from Clonetec, and mRNA of the large intestine was purchased from STRATAGENE. purchasing total RNA of liver and small intestine ** from Clonetec -- a law -- mRNA was prepared by the method. next, these every -- using an oligo dT primer from mRNA 0.12microg -- synthesis of single-strand cDNA -- a law -- it carried out in accordance with the method. SuperScriptIIRNAse HReverse Transcriptase (Invitrogen) was used as reverse transcriptase. mcd055 used for PCR -- the arrangement of a specific primer --

sense primer mcd055-F1 (5'-GTG TTG TCT ACT CTG TGG TGC- 3'). It is antisense primer mcd055-R1 (5'- AGC ATC TCC CTT CCC ATT CC- 3'). As a result of analyzing, mcd055 was revealed with a lung, liver, the heart, activation white corpuscles, white corpuscles, the spleen, and the trachea (drawing 1).

[0091]

Embodiment 3 Manifestation of meltable type MCD055 protein (MCD055 extracellular domain)

mcd055 gene was included in various expression vectors after amplification by PCR. In order to connect a histidine tag to the C-terminus side using expression vector pIVEX2.4bNde (Roche Diagnostics, Inc.) in order to connect a histidine tag to the proteinic N terminus side and to make it revealed, and to make it revealed.

********** pIVEX2.3 d (Roche Diagnostics, Inc.) was used. MCD055 protein uses and refines a nickel column, after making it revealed [according to a cell free system (Rapid Translation System:RTS) (Roche Diagnostics, Inc.)] in the form where the histidine tag was connected. Even the 339th Ser is revealed from the 317th Pro, and from the 1st Met to the 316th Val is made for an N terminus side to reveal from it as meltable type MCD055 protein in the film penetration part search by SOSUI for a film penetration part.

[0092]

Embodiment 4 Production of anti-MCD055 antibody

(1) In order to produce the antibody to preparation MCD055 partial peptide of partial peptide (peptide antigen) of MCD055, Partial peptide of MCD055 is dissolved [ml] in 10mg /with distilled water, and it mixes with 10mg/ml of maleimide-ized keyhole limpet hemocyanin (PIERCE) in equivalent amount. It desalts in NAP-10 column (Amersham Pharmacia Biotech) after a 2-hour reaction with room temperature. Protein concentration computes the used amount of KLH(s) using what was broken by volume.

[0093]

(2) Production of MCD055 antibody

(2-1) Production of a polyclonal antibody

In order to produce the rabbit polyclonal antibody to MCD055, it mixes the prepared peptide antigen each every [40micro / g], respectively, and may be 0.5 ml. Then, it mixes with an equivalent amount of Freund's complete adjuvant (DIFCO), and the regions-of-back hypodermic of a rabbit is medicated. What mixed tales doses with Freund's incomplete adjuvant (DIFCO) is prescribed for the patient in two weeks, it collects blood from the ear vein after two weeks, and an antiserum is prepared. Meltable type MCD055 similarly produced and refined in Embodiment 3 is prescribed for the patient by 30microg/body, and an antiserum is produced.

[0094]

(2-2) Production of a peptide antibody

Each peptide antigen 20mug which was chosen more nearly arbitrarily than the amino acid sequence of MCD055, and was prepared is dissolved in the physiological saline of 100microl, and the line of an equivalent amount of mixtures is carried out to Freund's complete adjuvant. The abdominal cavity of a BALB/c mouse 5-week old scalpel is medicated, after [two weeks] peptide antigen 20mug is dissolved in the physiological saline of 100microl, it mixes with Freund's incomplete adjuvant in equivalent amount, and the abdominal cavity is medicated similarly. It collects blood from the eyegrounds

after one week, and elevation of antibody titer is checked by Western blotting. That is, electrophoresis of the recombination MCD055 protein is carried out in a 4-20%SDS-polyacrylamide gel (TEFCO), and it transfers on a PVDF film in accordance with the procedure of Millipore Corp. It blocks with after-transfer 5% skim milk and the 0.076M phosphate buffer solution (pH 6.4) (following T-PBS and description) which contains Tween20 0.05%. The antiserum which collected blood is diluted with T-PBS which contains BSA 0.5% 500 times, and is made to react to the transferred PVDF film at 4 ** overnight. Membrane is washed 3 times by T-PBS, and a peroxidase-labeling anti-mouse immunoglobulin antibody (DAKO) is diluted with T-PBS which contains BSA 0.5% 500 times, and is made to react to membrane at room temperature for 1 hour. Then, ECL (Amersham Pharmacia Biotech) detects membrane after 3 times washing by T-PBS. Check elevation of antibody titer by the above operation, and a lymphocyte is separated from splenic cells three days after the last medication of an antigen, According to the Ando **** and the Chiba length / work "a guide to monoclonal antibody experiment operation" (Kodansha), cell fusion is performed after mixture with Sp2/O-Ag14 (ATCC No.CRL1581) using a polyethylene glycol. A hybridoma is chosen by a HAT medium and the hybridoma which is producing the target antibody in one week is screened.
[0095]
using 0.01M carbonic acid buffer solution (pH 9.5), and diluting [ml] the MCD055 extracellular domain which produced and refined in Embodiment 3 in 1 microg /-- an immuno plate (Maxisorb, NUNC) -- 50microl / well -- it adds. the 0.076M phosphate buffer solution (pH 6.4) (following PBS and description) which washes 5 times with ion exchange water after a 1-hour reaction at 37 **, and contains BSA 0.5% -- each -- it blocks by doing 100microl addition of at a well. next, a culture supernatant -- each -- after adding to a well and making it react at 37 ** for 1 hour, the physiological saline which contains Tween20 0.05% washes 3 times. diluting a peroxidase-labeling anti-mouse immunoglobulin antibody with PBS which contains rabbit serum 10% 1000 times -- each -- 50microl addition of is done at a well. the tetramethyl benzidine solution which washes 5 times with the physiological saline which contains Tween20 0.05% after a 1-hour reaction at 37 **, and contains hydrogen peroxide 0.01% -- each -- it adds to a well. A reaction is suspended with 0.5M sulfuric acid solution after a reaction for 10 minutes with room temperature. The absorbance of 450 nm is measured with a plate spectrophotometer (NJ-2100, Nihon Inter Electronics MEDDO). Based on this result, the cell reacted to MCD055 extracellular domain is chosen, and cloning is performed with limiting dilution. In ten days, it can screen similarly and the clone which produces the antibody reacted to MCD055 extracellular domain can be acquired. Cultivate the selected hybridoma after cultivation and by a Hybridoma-SFM medium (Invitrogen) by FCS/RPMI1640 medium (Invitrogen) 10%, it makes an antibody produce, and refines an antibody using a Prosep-A column (Millipore).
[0096]
(2-3) Production of anti-MCD055 monoclonal antibody
It mixes with Freund's complete adjuvant in equivalent amount, and MCD055 extracellular-domain protein 20mug refined to the abdominal cavity of a BALB/c mouse (a scalpel, 6-week old) is prescribed for the patient. Antigen 20mug is dissolved in a physiological saline after two weeks of first time medication, and the after-mixture abdominal cavity is medicated with an equivalent amount with Freund's incomplete

adjuvant. It checks in one more week by a procedure of the above-mentioned (2-2) description of elevation of antibody titer, and the last medication is performed. The abdominal cavity of a mouse is medicated with antigen 100mug, and a spleen is extracted in three days. A lymphocyte is separated from a spleen, it mixes by P3x63-Ag.8.U and 1, and 10:1, and cell fusion is performed using a polyethylene glycol. A hybridoma is chosen by a HAT medium and a hybridoma which is producing a contemptuous glance antibody for one week is screened. a procedure given in (2-2) -- anti-MCD -- a well is screened 055 antibody production, and cloning of the well reacted to MCD055 extracellular domain is carried out with limiting dilution, it screens again, and anti-MCD055 monoclonal antibody is acquired.

[0097]

Cultivate a hybridoma by a Hybridoma-SFM medium (Invitrogen) after cultivation by FCS/RPMI1640 medium (Invitrogen) 10%, an antibody is made to produce, and an antibody is refined using a Prosep-A column (Millipore).

[0098]

Production of the nature conversion stock of embodiment 5 MCD the original form of 055 shots

The transformant which reveals MCD055 stably is produced by the following procedures. that is, Into the Jurkat cell (clone E6.1;ATCC) of $5x10^{6}$ **. [the MCD055 manifestation plasmid of 20microg, and the Neomycin resistance gene manifestation plasmid (pcDNA3.1(+);Invitrogen) of 1microg] It introduces by the electroporation method. Electroporation is performed in 300V and 950micro F capacitance using a gene pulsar II system (BIO-RAD). Then, selective culture is performed in the medium containing G-418 [0.3mg/ml] (Invitrogen), and a G-418 tolerance clone is obtained. Next, the obtained tolerance clone is analyzed with the flow cytometry described in Embodiment 6, and the clone which has revealed MCD055 is obtained.

[0099]

Embodiment 6 MCD055 protein manifestation check

The manifestation in the cell membrane of the lymphocyte prepared from a spleen, peripheral blood, etc., the differential white blood count, and the Jurkat T-lymph cell which made this protein reveal is checked with a flow cytometer. The cell of 10^{6} ** which is the experimental target is made suspended to 0.1 ml of 0.1%BSA content PBS, the antibody to the extracellular domain of this invention protein of 1microg is added, and it is made to react at room temperature for 30 minutes. Carry out centrifugality of this sample, make 0.1% BSA content PBS of 100microl suspended after 3 times washing by 2-ml 0.1%BSA content PBS, and an FITC sign anti-mouse IgG antibody is 1microg Added, and it is room temperature and also is made to react for 30 minutes. After washing 3 times by BSA content PBS 0.1% again, it is re-suspended by 1-ml 0.1%BSA PBS, and the rate of the FITC positive cell contained in the re-suspension is detected and calculated with a flow cytometer.

[0100]

IL-2 production inhibiting-activities measurement of an embodiment 7 MCD055 protein antibody

Seeding of the Jurkat T-lymph cell of $2x10^{5}$ ** which made this invention protein reveal constantly is carried out to 96 hole plate. One evening is cultivated by the medium which added the 100 ng(s)/ml doxycycline, Then, the bead (the number of beads: Jurkat cell

number =1:1) which carried out the coat of the anti-CD-3 antibody and anti-CD28 antibody ml is added in 5 microg /, it cultivates for 48 hours, and IL-2 contained in a culture supernatant is measured by ELISA. The antibody to this invention protein is added simultaneously with addition of an anti-CD-3 antibody bead and anti-CD28 antibody, and influence on IL-2 production is considered.

[0101]

Phosphorylation inhibition reaction by an embodiment 8 MCD055 protein antibody this invention protein into the Jurkat cell of $1x10^{7}$ ** made to reveal by doxycycline addition An anti-CD-3 antibody bead (the number of beads: Jurkat cell number =1:1), Soluble anti-CD28 antibody After carrying out shot washing by PBS which adds 5 microg/ml and contains alt. vanadium acid sodium in 10 minutes, Buffer solution for the dissolution of 500microl (1% Triton.) X-100,150mM NaCl and 10mM. Tris-HCl pH 7.6, 5mM EDTA, and 1mM Alt. vanadium acid sodium, a 10microg/ml leupeptin, the 10microg/ml aprotinin, and 25microg/ml nitrophenyl p'-guanidino benzoate are added, and it settles in 30-minute ice. The phosphorylation ERK1/ERK2 is detected by transferring to a PVDF film, after performing SDS electrophoresis to the cell lysate obtained in this way, and performing western blotting using an anti-activity MAPK antibody. The antibody to this invention is added simultaneously with an anti-CD-3 antibody bead or anti-CD28 antibody, and influence on phosphorylation of ERK1/ERK2 is considered.

[0102]

Activity measurement of embodiment 9 extracellular domain

The peripheral blood lymphocyte (PBMC) prepared from two non-blood relationship persons' donor's (A, B) blood is prepared to $1x10^{6}$ cells/ml by fetal-bovine-serum addition RPMI1640 5%, The PBMC suspension of A origin and the PBMC suspension of B origin are added every [100micro / l] on 96 hole plate, and it cultivates for five days within 37 ** and a 5%CO_2 incubator. The MCD055 extracellular domain produced in Embodiment 3 is added 10 microg/ml simultaneously with addition of a lymphocyte. Then, by a medium, 20microl addition of the bromodeoxyuridine (BrdU) prepared to 100microM is done, and it is cultivated for 24 hours. After carrying out centrifugality for 10 minutes, settling 300 g of cells, removing a supernatant, adding FixDenat (Roche) of 200microl and settling for 30 minutes, FixDenat is removed, the peroxidase-labeling anti-BrdU reaction mixture of 100microl is added, and it settles at room temperature for 90 minutes. After washing 3 times by PBS, 100microl addition of the tetramethyl benzidine (TMB) was carried out, and it was made to color. 1M sulfuric acid of 25microl is added 5 to 30 minutes after coloring, a reaction is stopped, and fluorescence degree measurement of OD450nm is performed.

[0103]

[Effect of the Invention]

MCD055 of this invention is the protein which can become the cause to the onset or advance of a disease which an immune function depends unusually.

In development of the drug for prevention of an autoimmune disease, immune disorder, an allergic disease, the angiitis and hepatitis and the inflammatory disease that it is shocking septic, a tumor, etc., or treatment, it is very useful.

[0104]

Gene mcd055 can be used in the gene therapy as an antisense drug, and protein MCD055 is useful as a soluble protein drug by producing its itself or soluble fragment (an extracellular region and each domain). The antibody which has reactivity in MCD055 or its fragment, and a part of its antibody are useful as an antibody drug article which controls MCD055 function in the living body.
[Layout Table]

⟨110⟩ Mochida Pharmaceutical Co., Ltd.
⟨110⟩ Research Association for Biotechnology
⟨120⟩ A novel immunosuppressive receptor
⟨130⟩ 47681
⟨160⟩ 3
⟨170⟩ PatentIn Ver. 3.1

⟨210⟩ 1
⟨211⟩ 1239
⟨212⟩ DNA
⟨213⟩ homo sapiens
⟨400⟩

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〈210〉 2
〈211〉 413
〈212〉 PRT
〈213〉 homo sapiens
〈400〉 2
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RDGKFLHFSK ENQTLSMGAA TVQSRGQYSC SGQVMYIPQT FTQTSETAMV QVQELFPPPV 120
LSAIPSPEPR EGSLVTLRCQ TKLHPLRSAL RLLFSFHKDG HTLQDRGPHP ELCIPGAKEG 180
DSGLYWCEVA PEGGQVQKQS PQLEVRVQAP VSRPVLTLHH GPADPAVGDM VQLLCEAQRG 240
SPPILYSFYL DEKIVGNHSA PCGGTTSLLF PVKSEQDAGN YSCEAENSVS RERSEPKKLS 300
LKGSQVLFTP ASNWLVPWLP ASLLGLMVIA AALLVYVRSW RKAGPLPSQI PPTAPGGEQC 360
PLYANVHHQK GKDEGVVYSV VHRTSKRSEA RSAEFTVGRK FYHLCGGEMP AAQ 413

〈210〉 3
〈211〉 1997
〈212〉 DNA
〈213〉 homo sapiens
〈400〉 3
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atatacaaat atagcacata caattatgta cagtacacta tacttgataa tgataataaa 1980
caactatgtt actggtt 1997

[Brief Description of the Drawings]

[Drawing 1]It is a figure showing the organization manifestation profile of mcd055 by RT-PCR.

[Translation done.]